Androgen receptor-reduced sensitivity is associated with increased mortality and poorer glycaemia in men with type 2 diabetes mellitus: a prospective cohort study

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Introduction **Hypogonadism is associated with poorer glycaemic outcomes/increased all-cause and cardiovascular morbidity/mortality in type 2 diabetes mellitus (T2DM). Increasing CAG repeat number within exon-1 of the androgen receptor (AR) gene is associated with increased AR resistance/insulin resistance.**

Methods **We determined in a long-term 14-year follow-up cohort of 423 T2DM Caucasian men, the association between baseline androgen status/CAG repeat number (by PCR then Sequenom sequencing) and metabolic/cardiovascular outcomes.**

Results ***Metabolic outcomes*****: Lower total testosterone was associated with higher BMI (kg/m^2) at 14-year-follow-up: regression coefficient −0.30 (95% confidence interval −0.445 to −0.157), $P = 0.0001$. The range of CAG repeat number was 9-29 repeats. Higher CAG repeat number in exon-1 of the AR gene was associated with higher follow-up HbA1c2016 – each unit increase in CAG repeat-associated with an increment of 0.1% in HbA1C2016 ($P = 0.04$), independent of baseline testosterone.** ***Cardiovascular outcomes and mortality*****: At an average of 14-year-follow-up, 55.8% of hypogonadal men had died vs 36.1% of eugonadal men ($P = 0.001$). There was a 'u' shaped relation between number of CAG repeats and mortality. Twenty-one CAG repeats were associated with an up to nearly 50% lower mortality rate than <21 CAG repeats and >21 CAG repeats – independent of baseline testosterone level.**

Conclusion **A higher number of CAG repeats at the AR gene associates with higher future HbA1c. There was a 'u' shaped relation between CAG repeat number and mortality rate. Determination of CAG repeat number may become part of assessment of androgen status/its consequences for men with T2DM.** ***Cardiovasc Endocrinol Metab*** **10: 37–44**

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Learning points

(1) Hypogonadism has been associated with poorer glycaemic outcomes and increased cardiovascular morbidity/mortality in type 2 diabetes mellitus (T2DM).
(2) An increasing CAG repeat number within exon 1 of the androgen receptor gene polymorphism is associated with increased androgen receptor insensitivity.
(3) We here investigated the link between CAG repeat number and outcomes in T2DM men.
(4) There was a 'u' shaped relation between the number of CAG repeats and mortality such that 21 CAG repeats was associated with an up to 58% lower mortality rate than <20 CAG repeats and >21 CAG repeats.
(5) CAG repeat number may become part of the assessment of androgen status for men in the future but further work in this area will be required.

In men with type 2 diabetes mellitus, a higher number of CAG repeats at the androgen receptor gene is associated with a higher future HbA1c. We found a 'u' shaped relation between CAG repeat number and mortality rate, such that 21 CAG repeats was associated with an up to

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58% lower mortality rate than <20 CAG repeats and >21 CAG repeats. independent of baseline testosterone level.

Introduction

Type 2 diabetes mellitus (T2DM) is a growing health issue around the world. In 2018, it was estimated that there were more than 500 million prevalent cases of T2DM worldwide with the prevalence comparable between high- and low-income countries [1]. Several studies have demonstrated a higher prevalence of hypogonadism in T2DM men than those with normal glucose regulation and with type 1 diabetes (T1DM) [2–5]. In men with T2DM, low serum levels of total testosterone have been associated with a more adverse cardiometabolic risk profile and with increased mortality [3,6–8], as also recently described in a long prospective UK cohort [9].

The androgen receptor (AR) mediates the peripheral effects of testosterone. The main mechanism of action for the AR is direct regulation of gene transcription. After the binding of an androgen to its receptor, a conformational change occurs, causing the dissociation of heat shock proteins, translocation into the nucleus, and dimerization [10]. The AR dimer binds to a specific sequence of DNA, known as an androgen response element, thereby up- or downregulating specific gene transcription [6,11]. Testosterone effect occurs via the AR, through its more active metabolite, dihydrotestosterone (DHT) [10,12], in which it is converted by the enzyme 5-α reductase [3].

Testosterone may also act by a pathway that entails the rapid activation of kinase-signaling cascades and the modulation of intracellular calcium levels working directly on the calcium channel. This is a nongenomic effect [6,10].

The AR gene is composed of eight exons and is located on X chromosome at q11-q12. Exon 1 of the AR gene contains a polymorphic sequence of CAG repeats, which usually varies in number from 10 to 35, and which encodes polyglutamine stretches of the AR transactivation domain [6]. The evidence suggests that the CAG number is negatively correlated with the transcriptional activity of the AR [10,13].

Men affected by the Kennedy syndrome have more than 40 CAG repeats. The condition is characterized by decreased virilization, testicular atrophy, reduced sperm production, and infertility [14]. A shorter CAG repeat sequence has been associated with prostate disease, specifically cancer and benign hypertrophy, improved seminal parameters, and improved bone mineral density (BMD) [15,16]. Longer CAG repeat length in people with T2DM has been associated with greater waist circumference, serum leptin, and HDL cholesterol [6,13].

Our primary hypothesis was that the previously described relation between hypogonadism and fewer favorable outcomes in T2DM men is modulated by the number of CAG repeats at the AR gene with regard to long-term metabolic outcomes and mortality.

Aims

To measure initial testosterone, sex hormone binding globulin (SHBG), and testosterone receptor gene CAG repeat number and to ascertain the relation between initial androgen status and the number of the CAG repeats with (a) change in HbA1c/BMI over time (b) cardiovascular events, and (c) all-cause mortality.

Methods

The Salford prospective diabetes cohort provides a unique opportunity to determine how the relationship between androgen profile, and the cardiometabolic outcome is modulated by the number of CAG repeats of the AR. The cohort of mostly Caucasian individuals with T2DM was established in 2002. Follow-up data were available on 423 men. Individuals were recruited consecutively from outpatient clinics and general practitioner (GP) surgeries. We have serum samples and extracted DNA for 423 men collected between 2002 and 2004 with detailed cardiometabolic phenotypic follow-up data including mortality data on all these men up to the end of 2016. Furthermore, we have details as to all medication changes including initiation of testosterone therapy with subsequent testosterone levels in which this was done as part of routine care.

Participants donated a blood sample for DNA extraction and circulating hormone/biomarker measurement. Blood samples were collected only once (at baseline)/initial screening and individuals were asked to attend fasted (no food and only water from 2300 on the day before. In relation to androgen status, only a baseline blood test was taken. The project described here is part of a larger long-term naturalistic T2DM cohort study which aims to provide genetic and epigenetic data to inform our understanding of the outcomes for T2DM patients in a longer-term follow-up model. We have explicit written consent from the participants to obtain follow-up health outcome data for them. Ethical permission was obtained.

We have recently obtained (with full ethics permission) the complete cardiometabolic data set for men from 2002 up to the end of 2016 including mortality data. This data set includes changes in weight, BMI, blood pressure, and HbA1c/renal indices/lipid profile, plus cardiovascular complications/neoplasia from GP and hospital-coded diagnoses and death. Cardiovascular events were defined as stroke, myocardial infarction (MI), angina, and coronary revascularization. Fifty two men did not have available follow-up data as they had left Salford, with the consequence that their health records were no longer accessible. CAG analysis was not performed on samples from these men.

We determined baseline androgen status by measuring serum testosterone, using tandem mass spectrometry at the University Hospital of South Manchester. Also, in collaboration with the department of Oncology and Metabolism at the University of Sheffield, the number of CAG repeats for the testosterone receptor gene was determined using whole blood-derived DNA. The related methodology is summarized below.

Assays

Hormone assays

Baseline androgen profile was determined by measuring serum total testosterone using tandem mass spectrometry at University Hospital of South Manchester [17], and SHBG, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) by immunoassay at the same laboratory.

Free testosterone was estimated using the calculator available at http://www.issam.ch/freetesto.htm which is based on the Vermeulen Equation [18]. HbA1c and lipid profile were determined at Salford Royal Hospital on the Menarini 9210 Premier automated analyser (boranate affinity and high performance liquid chromatography), (Menarini, Wokingham Berkshire, UK).

Determination of the CAG repeat number (423 individuals)

DNA that had already been extracted from whole blood was subjected to PCR for 423 individuals to amplify the region of the AR gene containing AR CAG. PCR preparation, primers, and conditions were undertaken as described in a previous study [6].

Statistical analysis

The CAG distribution was plotted using Kernel density estimation. Kernel density estimation is a fundamental data smoothing solution where inferences about the population are made, based on a finite data sample.

To explore whether baseline data and characteristics were the same across different groups of CAG repeats, one-way ANOVA was applied. The number of CAG repeats was included as a categorical predictor variable. Testosterone at baseline was included as either continuous predictors or as dichotomous variables [normal (>12 nmol/L)/borderline (8–12 nmol/L)/low (<8 nmol/L)]. Initially, single predictors were fitted to determine whether the continuous or dichotomous variables were better at predicting HbA1c. Then a single measure for testosterone/free testosterone was included to determine the best possible prediction of mean HbA1c/BMI levels over time, including BMI and HbA1C at last follow-up. Interactions between the predictors and time were then added to determine whether there was an effect on the rate of change of HbA1c over time. Finally, the number of CAG repeats was added to the model to determine whether the effect of androgen status is modulated by CAG repeat number.

A similar modeling procedure was followed to determine the combined effects of CAG repeats and testosterone level on mortality and on the risk of cardiovascular events. Logistic regression, including age as a covariate, was used to determine the influence of CAG repeat number on mortality as the outcome variable.

Results

Mean (09:00 a.m. to 11:00 a.m.) total testosterone was 13.7 ± 5.8 nmol/L. A total of 154 (28.0%) of men had a low total testosterone (defined as total testosterone <10 nmol/L). We chose 10 nmol/L as the mid-point of the laboratory reference range for borderline biochemical hypogonadism (8–12 nmol/L) [19]. Sixty four men were prescribed testosterone replacement either for a short period or long term over the follow-up period. Analysis of the outcomes for these individuals is the subject of a separate study.

The mean age of the men was 59.8 ± 12.5 years. In total, 175/423 men died during follow-up. The all-cause mortality rate was higher in patients with lower total testosterone compared to normal baseline total testosterone (5.2% vs 2.9% per year, $P < 0.0001$) and also for lower free testosterone. Over the whole follow-up period, 36.3% men with normal baseline total testosterone at baseline died vs 55.9% of those who were hypogonadal at baseline.

Mean baseline BMI (2002) was 29.90 (95% confidence interval (CI) 29.45–30.45) with a decrease in mean BMI in those still available for follow-up to 29.40 (95% CI 28.60–30.20). Mean baseline HbA1c (2002) was 8.00% (95% CI 7.85–8.17%) (63.9 mmol/mol) increasing to a mean HbA1c (2016) of 8.23% (95% CI 8.05–8.40) (66.4 mmol/mol) at the last follow-up.

Baseline data by categories of CAG repeats are given in Table 1. The highest (n > 23) vs lowest quartile of CAG repeats (n < 20) associated with a 1.1 nmol/L higher baseline testosterone level (F = 1.94, $P = 0.02$).

Metabolic outcomes

Lower total testosterone was associated with higher BMI (kg/m^2) at 14-year-follow-up: regression coefficient –0.30 (95% CI –0.445 to –0.157), $P = 0.0001$.

For the 423 individuals undergoing CAG repeat analysis, the distribution of CAG repeats is shown in Fig. 1. The range of CAG repeat number was from 9 to 29 repeats.

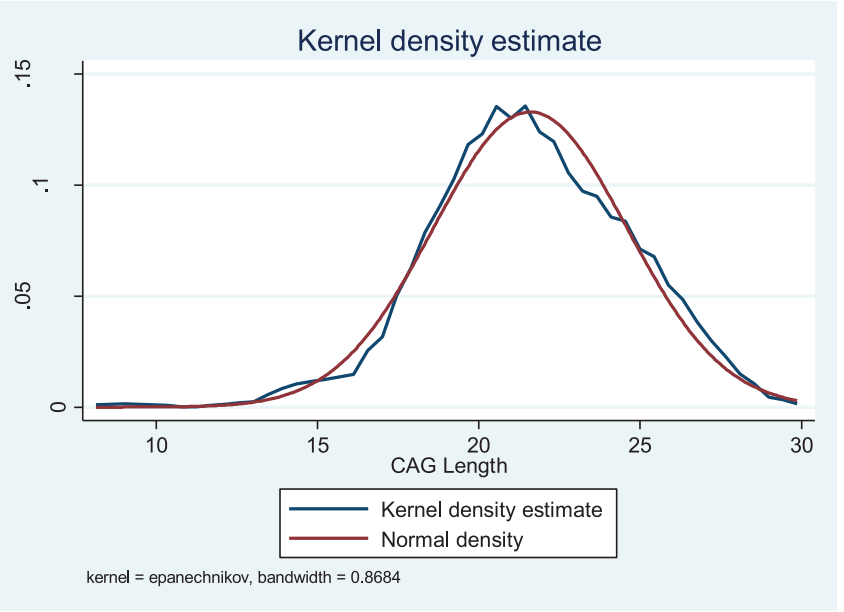
A higher CAG repeat number in exon 1 of the AR gene was associated with higher follow-up BMI 2016 after a mean 14-year-follow-up in 2016 such that each unit increase in CAG repeat associated with an increment of 0.43 in BMI 2016 ($P = 0.018$) (Fig. 2); and also higher HbA1c 2016

Table 1 **Baseline variables (2002) for the Salford diabetes cohort men by number of CAG repeats**

	CAG repeat number ≤20	CAG repeat number 21	CAG repeat number 22–23	CAG repeat number ≥24	
	N = 150	N = 66	N = 86	N = 121	F, p for difference
Age (years)	60.8 (58.1–63.5)	58.8 (55.2–62.3)	56.7(53.2–60.2)	58.8 (56.0–61.5)	F 1.25, pNS
BMI (kg/m^2)	30.4 (29.2–31.6)	28.0 (26.8–29.3)	28.6 (27.2–30.0)	30.4 (29.1–31.7)	F 3.32 $P = 0.02$
Systolic BP (mmHg)	138 (134–142)	134 (128–140)	134 (129–139)	137 (1340141)	F 0.85, pNS
Diastolic BP (mmHg)	77 (74–79)	76 (72–80)	74 (71–77)	77 (75–80)	F 0.82 pNS
Hba1c (%)	8.45 (7.98–8.92)	8.43 (7.78–9.10)	8.22 (7.80–8.64)	8.21 (7.84–8.59)	F 0.30 pNS
HbA1C (mmol/mol)	68.9 (63.7–74.0)	68.6 (61.5–76.0)	66.3 (61.7–70.9)	66.2 (62.2–70.4)	F 0.30 pNS
Total cholesterol (mmol/L)	4.80 (4.53–5.07)	4.65 (4.26–5.04)	4.78 (4.46–5.10)	4.70 (4.47–4.93)	F 0.23 pNS
HDL cholesterol (mmol/L)	1.17 (1.10–1.24)	1.25 (1.09–1.40)	1.30 (1.12–1.48)	1.22 (1.12–1.33)	F 0.92 pNS
Creatinine (μmol/L)	100.8 (93.2-108.3)	99.9 (93.3–106.5)	94.8 (88.6–101.1)	96.0 (90.7–101.3)	F 0.73 p NS
Testosterone (nmol/L) *	13.6 (12.4–14.8)	14.6 (13.1–16.0)	14.2 (12.6–15.8)	14.9 (13.5–16.3)	F 1.94 p 0.02
SHBG (nmol/L) *	41.1 (37.5–44.8)	47.1 (41.3–52.9)	41.5 (36.9–46.0)	41.2 (36.7–45.8)	F 1.34 pNS
Free testosterone (pmol/L) *	245 (225–266)	244 (222–266)	250 (229–271)	271 (250–293)	F 1.41 pNS

Numbers of men shown in brackets for each CAG category. All data are arithmetic means [95% confidence interval (CI)] unless otherwise stated.
Geometric means are denoted by *

Fig. 1



Frequency distribution of CAG repeat number.

(Fig. 3) – each unit increase in CAG repeat associated with an increment of 0.1% in HbA1C 2016 (P = 0.04). These relations were independent of baseline testosterone.

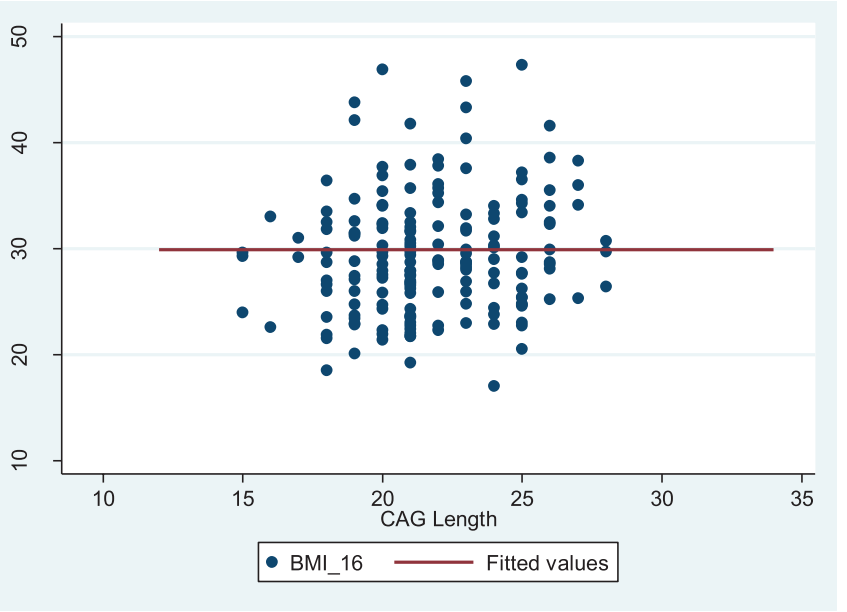
Cardiovascular outcomes and mortality

There was no relation between CAG length and the rate of occurrence of MI, coronary artery revascularization, angina, or stroke.

At an average 14-year-follow-up, 55.8% of hypogonadal men had died vs 36.1% of eugonadal men (P = 0.001). In total, 175/423 (41.4%) men died. There was a 'u' shaped relation between the number of CAG repeats and mortality such that 21 CAG repeats was associated a lower mortality rate than <21 CAG repeats and >21 CAG repeats (Fig. 4). Adjusted logistic regression (including adjustment for age) indicated that the presence of 21 CAG repeats reduced the risk of death by 45% compared with <21 CAG repeats (odds ratio 0.55 (95% CI 0.28–0.92), P = 0.02; and that >21 repeats increased the risk of death by 48% (odds ratio 1.48 (95% CI 1.08–2.37, P = 0.015).

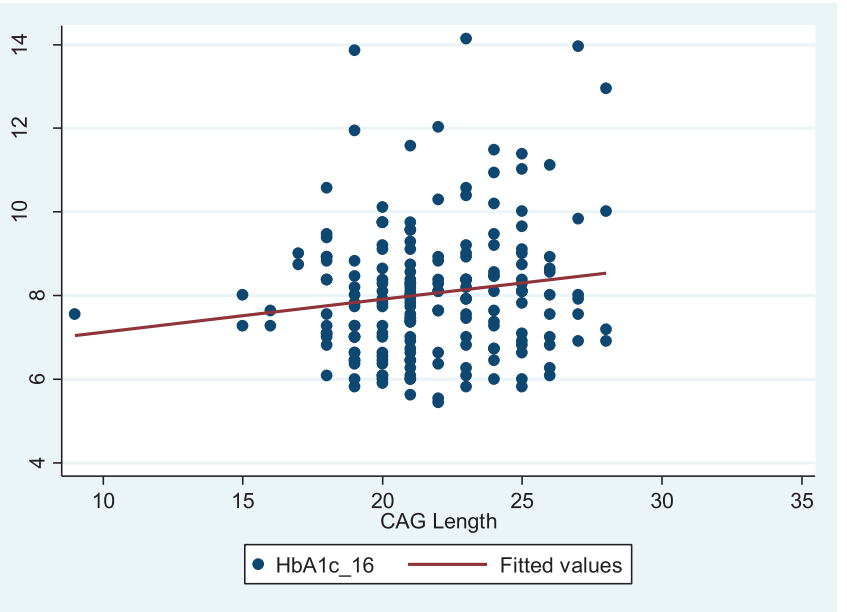
Thus, we found an optimal number of CAG repeats in relation to mortality rate. This relation was independent of baseline testosterone level.

Fig. 2



Relation between CAG repeat number and BMI 2016. Each unit increase in CAG repeat associated with an increment of 0.43 in BMI 2016 ($P =$ 0.018).

Fig. 3

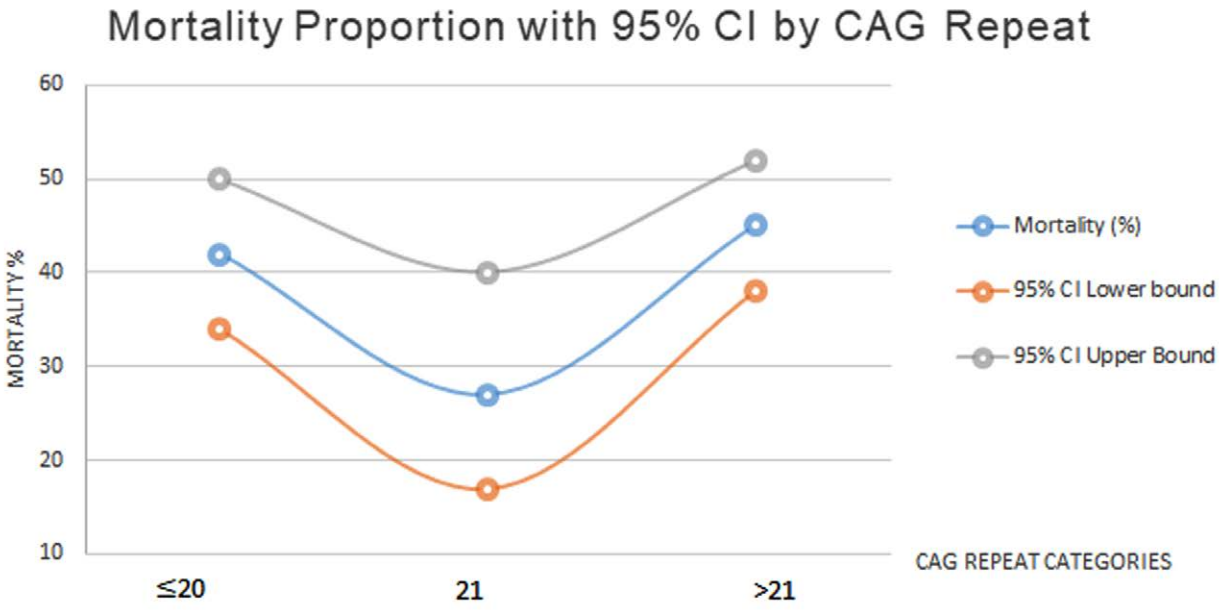


Relation between CAG repeat number and HbA1c 2016. Each unit increase in CAG repeat associated with an increment of 0.1% in HbA1C 2016 ($P = 0.04$).

Discussion

We have determined a 'u' shaped relation between CAG repeat number and mortality rate in this prospective cohort study. This has not been described previously in such a long-term follow-up study and points to an optimum number of CAG repeats in terms of how the CAG repeat number influences AR function and responsiveness to testosterone, both with a lower and higher

Fig. 4



Relation between GAC repeat number and mortality rate. Adjusted logistic regression indicated that the presence of 21 CAG repeats reduced the risk of death by 45% compared with <21 CAG repeats [odds ratio 0.55 (95% CI 0.28–0.92), $P = 0.02$]; and that >21 repeats increased the risk of death by 48% [odds ratio 1.48 (95% CI 1.08–2.37, $P = 0.015$]. CI, confidence interval.

number of CAG repeats than 21 potentially associated with greater AR resistance to testosterone.

We also found that a higher number of CAG repeats at the testosterone receptor gene is associated with a higher future BMI and HbA1c. This is the longest duration study in T2DM men to have shown this and indicates that CAG repeat number should potentially be taken into account in interpreting total testosterone level in relation to modulation of longer-term health outcomes in men.

Reasons for the 'u' shaped relation between CAG repeat number and mortality rate may include influences of a low or high CAG repeat number on long-term changes (increase) in HbA1c, BMI, and HDL cholesterol or on other determinants of cardiovascular outcome.

It is also relevant to note that there was a relation between CAG repeat number and total testosterone at baseline with the highest (n > 23) vs lowest quartile of CAG repeats (n < 20) associated with a 1.1 nmol/L higher baseline testosterone level, potentially as a compensation for the degree of testosterone resistance conferred by the increased number of CAG repeats.

Ethnic variation in disease prevalence can inform our understanding of the factors that drive these differences. Differences in the CAG repeat length of the AR gene exist between African, Caucasian, and Asian populations [20]. The CAG allele expansion in African men was reported at between 18 and 20 [21]. In contrast, Caucasian and Asian populations have been shown to have a longer CAG expansion, where the mean number of CAG repeats is, respectively, 21–22 in Caucasians [22] and 23 in Asians [16].

A body of evidence exists indicating that hypogonadism is associated with a more adverse metabolic profile and cardiovascular risk [9,23]. A lower circulating testosterone level in illness caused by cytokine suppression of the hypothalamic-pituitary-testicular axis may reflect a biological protective effect on the species and not the individual. Evidence does support a beneficial action of testosterone on atherosclerosis and its clinical consequences [24], however, this has been disputed in the literature [25]. Nevertheless, this is the longest duration prospective study to describe the potential impact of CAG repeats length of the AR on cardiovascular risk and lipid profile. The mortality rate in this study is similar to that described in the prospective BLAST study [8] and by Muraleedharan *et al.* in the untreated arm of their study of testosterone therapy in hypogonadal T2DM men [7].

A previous study on 1859 men aged 20–79 years showed no direct correlation between CAG repeat length in the AR coding region and cardiometabolic risk factors [26]. Other authors also found a neutral effect of the length of AR gene polyglutamine tract on lipid levels [27,28]. However, in T2DM longer CAG repeat number was associated with HDL cholesterol levels but not with total or LDL cholesterol levels [13]. Nevertheless, a shorter CAG repeat of

the AR gene was found by Alevizaki *et al*. to be associated with more severe coronary artery disease (CAD) [27].

Important functions of testosterone in modulating adiposity, insulin resistance, and T2DM have been postulated [3,13]. It has been known for some time that total testosterone and SHBG levels are lower in men with T2DM compared with healthy controls [29] and lower than in men with T1DM [30]. In some men, the lower SHBG resulted in estimated free testosterone not itself being lower in the T2DM men. A longer AR CAG repeat was associated with increased body fat and leptin levels in a study of 106 healthy men [31] and in a study of 233 men with T2DM [6]. The latter study demonstrated that increased central adiposity as measured by waist circumference was associated with a less sensitive AR. Longer AR CAG sequences have also been associated with higher serum insulin levels in healthy men [21] and with obesity and leptin levels, although they were not shown to correlate with HbA1c levels in our study.

There were insufficient individuals in this study to look at the relation between testosterone therapy and cardiovascular events/CAG repeat number in a way that would provide a definitive answer to the question. This is something that we plan to do once we have a larger cohort as the next step in our work.

Strengths and limitations

A strength of the study is the duration of follow-up. A limitation of the study is that CAG analysis was not carried out on all the original participants. However, only 52 men did not have follow-up data. CAG analysis was not performed on samples from these men. A further limitation is that the follow-up data was obtained from GP records and therefore is limited to what data was routinely recorded. Also, there were not sufficient follow-up testosterone levels to look prospectively at trends in testosterone over time. Finally, the Salford area population is predominantly of Caucasian ethnicity, so we cannot draw conclusions concerning other ethnic groups from this study in relation to AR status.

Conclusion

In the near future, determination of AR CAG polymorphisms may become of clinical relevance because of the theoretical possibility of identifying subjects more or less at risk for various disorders and more or less responsive to testosterone therapy. Furthermore, the study of CAG repeat length could allow us to individualise testosterone therapy, according to the number of CAG repeats.

A greater understanding of the interaction between CAG repeat number and circulating testosterone level adds further to our understanding of the endocrine factors that determine longer term health outcomes in men with T2DM.

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Conflicts of interest

There are no conflicts of interest.

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